When Hormone Defects Cannot Explain It: Malformative Disorders of Sex Development

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The birth of a baby with malformations of the genitalia urges medical action. Even in cases where the condition is not life-threatening, the identification of the external genitalia as male or female is emotionally essential for the family, and genital malformations represent one of the most stressful situations around a newborn. The female or male configuration of the genitalia normally evolves during fetal life according to the genetic, gonadal, and hormonal sex. Disorders of sex development occur when male hormone (androgens and anti-Müllerian hormone) secretion or action is insufficient in the 46,XY fetus or when there is an androgen excess in the 46,XX fetus. However, sex hormone defects during fetal development cannot explain all congenital malformations of the reproductive tract. This review is focused on those congenital conditions in which gonadal function and sex hormone target organ sensitivity are normal and, therefore, not responsible for the genital malformation. Furthermore, because the reproductive and urinary systems share many common pathways in embryo-fetal development, conditions associating urogenital malformations are discussed.

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Key words: uterine and vaginal malformations; cloacal and bladder exstrophy; aphallia; hypospadias; penoscrotal transposition

Introduction

The birth of a baby with malformations of the genitalia urges the medical team to take prompt but cautious actions. On one hand, it may be the apparent sign of a life-threatening condition, like congenital adrenal hyperplasia or polymalformative syndromes, severely affecting renal function. On the other, even if the condition is not life-threatening, the identification of the external genitalia as male or female is emotionally essential for the family, and genital malformations represent one of the most stressful situations around a newborn.

The female or male configuration of the genitalia normally evolves during fetal life according to the genetic, gonadal, and hormonal sex, as has been established for decades. Disorders of sex development (DSD) occur when male hormone secretion or action is insufficient in the 46,XY fetus or androgens are excessive in the 46,XX fetus, as will be briefly reviewed later. However, sex hormone defects during fetal development cannot explain all congenital malformations of the reproductive tract. This review focuses on those congenital conditions in which gonadal function and sex hormone target organ sensitivity are normal and, therefore, not responsible for the genital malformation. Furthermore, because the reproductive and urinary systems share many common pathways in embryo-fetal development, we will particularly describe conditions associating urogenital malformations.

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Normal Fetal Sex Development

The chromosomal sex is defined at the moment of fertilization by the sex chromosome present in the spermatozoon. Yet, the embryo remains sexually undifferentiated for approximately 7 weeks in humans. Thereafter, the differentiation of the gonads into testes secreting male hormones is determinant for the subsequent fate of the internal and external genitalia (Fig. 1).

The genital system shares its embryologic origin with most structures of the urinary system and with some of the hindgut. These features will be briefly reviewed to settle the basis for the understanding of complex congenital malformations involving genital, urinary, and digestive systems, and their pathogenic differences with those limited to sex-organ anomalies.

THE UNDIFFERENTIATED STAGE OF SEX DEVELOPMENT

During this stage, no anatomic, histologic, or physiologic distinction can be made between the XX and the XY embryo. For the sake of simplicity, separately the initial development of the urogenital ridge and the subsequent formation of the primordia of the gonads, internal genital ducts, urogenital sinus, and external genitalia are described.

The urogenital ridges. The urogenital ridges develop during the fourth week after fertilization; they are formed by

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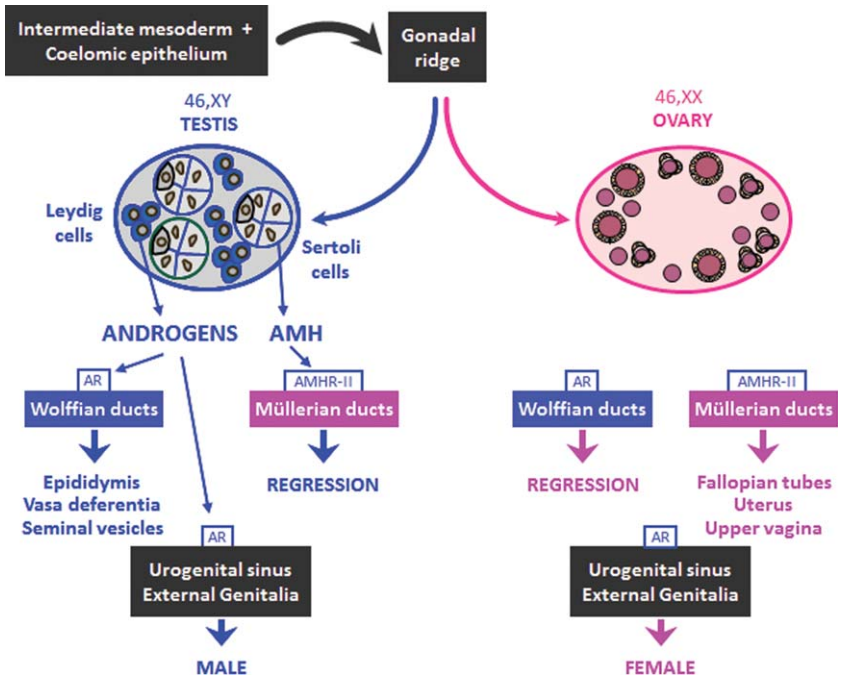


FIGURE 1. *Fetal sex differentiation. The testis secretes two distinct hormones: androgens, which act on the androgen receptor (AR), drive the differentiation of the mesonephric (Wolffian) ducts and the virilization of the urogenital sinus and the anlagen of the external genitalia, and anti-Müllerian hormone (AMH), which is responsible for the regression of the paramesonephric (Müllerian) ducts, signaling through its Type 2 receptor (AMHR-II). In the female embryo, the ovaries do not secrete androgens or AMH; therefore, Wolffian ducts regress, Müllerian ducts give rise to the uterus, Fallopian tubes, and upper vagina, the urogenital sinus gives rise to the lower part of the vagina, and the external genitalia feminize.*

intermediate mesoderm covered by coelomic epithelium of mesodermal origin, and are the common precursors of the urinary and genital systems and of the adrenal cortex. In the intermediate mesoderm, three slightly overlapping systems develop in a cranial-to-caudal sequence: the pronephros, mesonephros, and metanephros. The pronephros is rudimentary and nonfunctional. During the fifth week, each urogenital ridge divides into a urinary ridge and an adrenogenital ridge. In the urinary ridge, located laterally, the metanephros gives rise to most of the renal parenchyma, that is, the Bowman's capsule of the renal corpuscle, the proximal convoluted tubule, the loop of Henle, and the distal convoluted tubule.

The gonadal primordial. Each adrenogenital ridge, located medially to the ipsilateral urinary ridge, is a thickening of the coelomic epithelium covering the mesonephric mesoderm. The interaction of both tissues contributes to the formation of the gonad and adrenal cortex. The gonadal primordium becomes separated from the adrenal primordium by the end of the fifth week, is subsequently colonized by the primordial germ cells of extragonadal origin, and remains sexually undifferentiated until the seventh week. The gonadal primordia are bipotential and can develop into ovaries or testes.

Several general transcription factors play an important role in the stabilization of the intermediate mesoderm and the formation of the urogenital ridges, and the gonadal and renal primordia. Most of these ubiquitous factors are also essential for the development of other vital embryonic structures, and impaired expression in early embryogenesis is certainly lethal (reviewed by Rey and Josso, 2013).

The urinary and genital ducts. Wolffian ducts. In each genital ridge also develop the primordia of the urinary and genital ducts. From the fourth week, the mesonephric or Wolffian duct can be observed in the mesonephros as a longitudinal tubule that opens into the caudal part of the hindgut, the cloaca. The Wolffian ducts are unipotential and originate the male genital excretory system (Fig. 2).

A single outgrowth, known as the ureteric bud, evaginates from the Wolffian duct close to its entrance to the cloaca and grows dorsally, in response to inductive signals from metanephric mesenchyme involving GREMLIN1, BMP4, and BMP7 (Gonçalves and Zeller, 2011). The bud gives rise to the ureter, which penetrates the metanephros, dilates forming the primitive renal pelvis, and splits into cranial and caudal portions, the future major calyces. Subsequent divisions give rise to the minor calyces and the collecting tubules, which fuse with the distal convoluted tubules, of metanephric origin. HNF1β, a transcription factor expressed in the developing liver and pancreas, is also expressed in Wolffian and Müllerian ducts, and is crucial for the development of Wolffian duct derivatives, including the ureteric bud and nephrogenesis (Lokmane et al., 2010). PAX2 and PAX8 proteins are required for mesenchymal–epithelial conversion, which is essential for the formation of Wolffian ducts (Bouchard et al., 2002). LIM1 (Kobayashi and Behringer, 2003) and EMX2 (Miyamoto et al., 1997) are also necessary for Wolffian and Müllerian duct development subsequently to PAX2 induction.

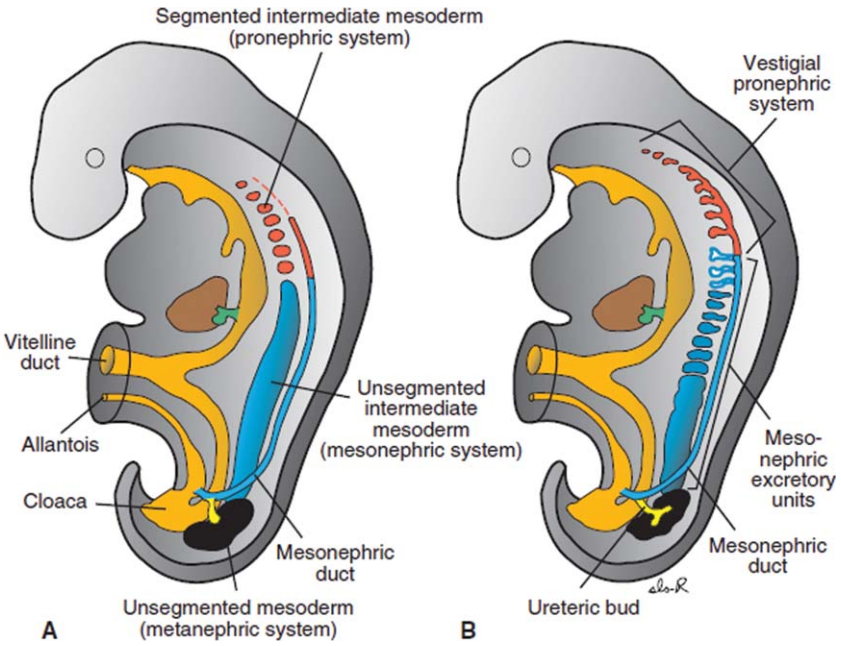


FIGURE 2. *Urogenital structures deriving from the intermediate mesoderm and cloaca. The intermediate mesoderm gives rise to the mesonephros, which can be divided into the pronephros, mesonephros, and metanephros. The pronephros regresses, the mesonephros forms urogenital ridge, including the mesonephric (Wolffian) ducts, whereas the metanephros is the main precursor of the kidney. The distal part of the hindgut and the allantois form the cloaca: its ventral portion is the urogenital sinus, where the Wolffian ducts open. Reprinted with permission from: Sadler TW. Langman's medical embryology. 12th ed. 2012. ©Lippincott Williams & Wilkins, a Wolters Kluwer business.*

Retinoic acid signaling also plays a role in Wolffian and Müllerian duct development, as well as in development of numerous other organs (Mendelsohn et al., 1994). RET signaling is involved in multiple aspects of early Wolffian duct development (Hoshi et al., 2012).

Müllerian ducts. After the Wolffian ducts are formed, on the anterolateral surface of each urogenital ridge arise the paramesonephric or Müllerian ducts as longitudinal invaginations of the coelomic epithelium through the mesonephroi. The Müllerian ducts are unipotent and originate the female genital duct system (Fig. 3). They open cranially into the abdominal cavity. Caudally, each Müllerian duct runs laterally to the Wolffian duct, then crosses it ventrally to reach the midline and comes in contact with the contralateral Müllerian duct. Initially separated by a septum, the ducts subsequently fuse to form the uterine canal. The caudal end of the fused ducts projects into the posterior wall of the urogenital sinus, as the Müllerian tubercle, located medially to the paired openings of the Wolffian ducts. The development of the Müllerian ducts occurs in three phases (Orvis and Behringer, 2007). Initially, there is a placode-like thickening of the coelomic epithelium, characterized by the expression of LIM1 and anti-Müllerian hormone receptor type II (AMHR-II) (Zhan et al., 2006; Arango et al., 2008). In the second phase, these primordial Müllerian cells invaginate from the coelomic epithelium to reach the Wolffian duct, induced by WNT4 expression from the mesonephros or coelomic epithelium (Vainio et al., 1999; Kobayashi et al., 2004). These phases of Müllerian duct development are Wolffian duct independent (Kobayashi and Behringer, 2003; Carroll et al., 2005). Upon contact with the Wolffian duct, the third or elongation phase begins, resulting in proliferation and caudal migration. Close contact with the Wolffian duct is essential for Müllerian duct growth. When Wolffian ducts do not develop, Müllerian duct truncation occurs. Although Wolffian ducts do not contribute cells to the elongating Müllerian ducts (Guioli et al., 2007; Orvis and Behringer,

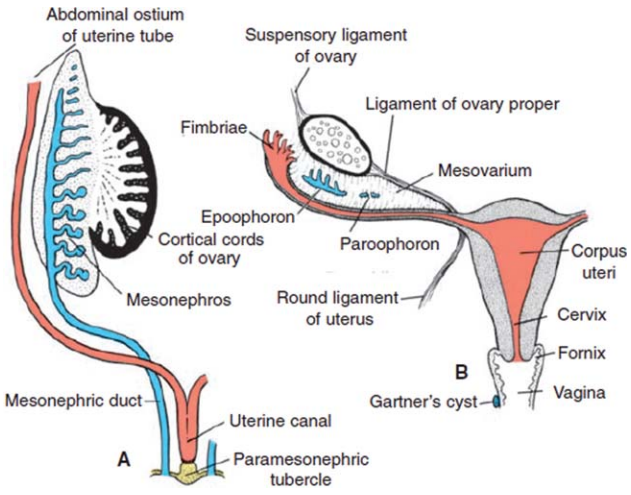


FIGURE 3. *Female genital ducts derived from the paramesonephric (Müllerian) ducts. Each paramesonephric duct bend toward the midline and reaches the contralateral one to fuse and form the uterine canal. Later in fetal life, they form the Fallopian tubes, the uterus, and the upper third of the vagina. The epoophoron, the paroophoron, and the Gartner's cysts are remnants of the mesonephric (Wolffian) ducts. Reprinted with permission from: Sadler TW. Langman's medical embryology. 12th ed. 2012. ©Lippincott Williams & Wilkins, a Wolters Kluwer business.*

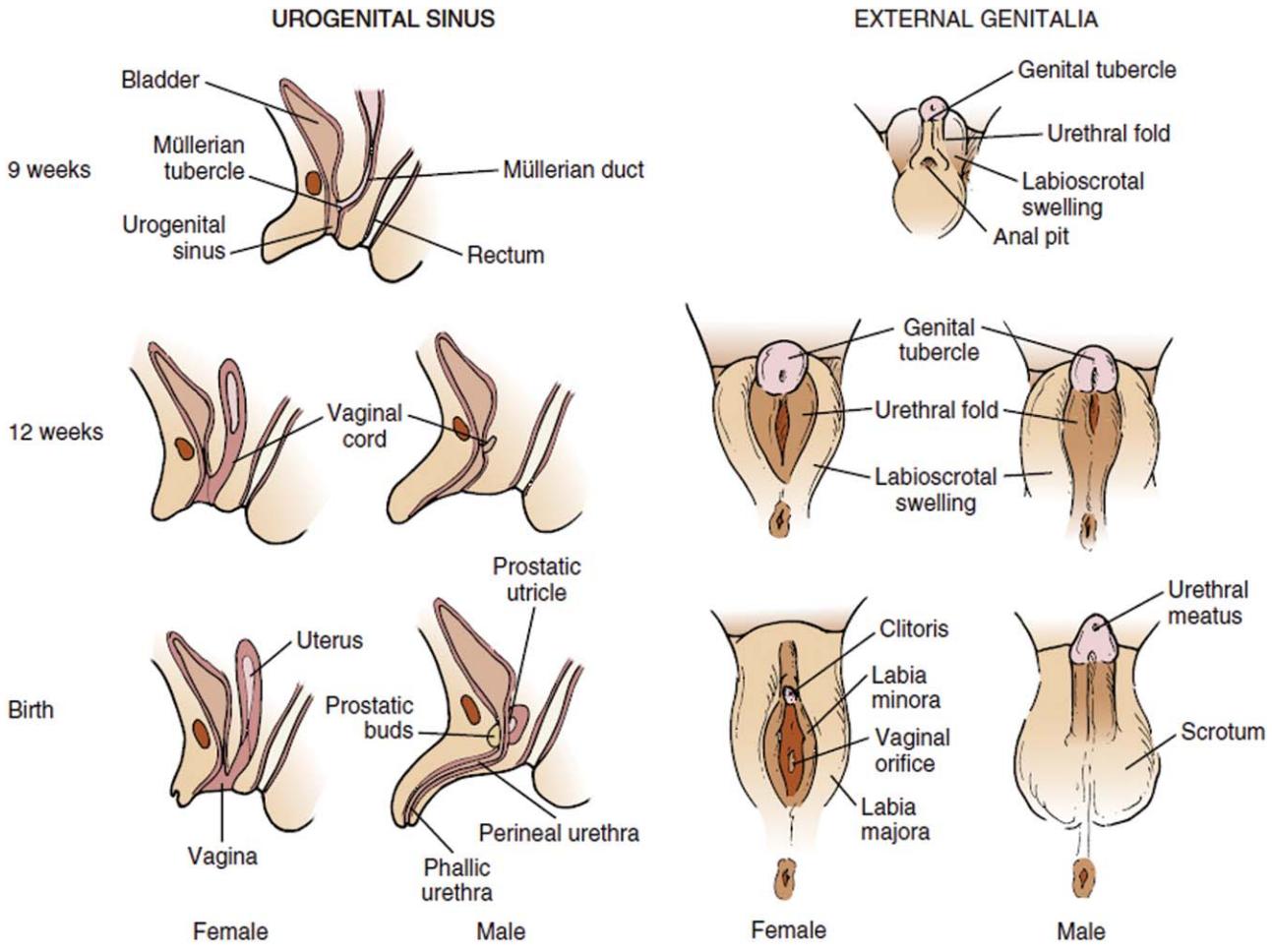


FIGURE 4. *Sex differentiation of urogenital sinus (**left**) and external genitalia (**right**). Modified with permission from: Rey R, Josso N. Sexual differentiation. In: De Groot LJ, editor. Endotext.org. Updated February 8, 2013. Available at: http://www.endotext.org/pediatrics/pediatrics7/pediatricsframe7.htm. 2000–2013. ©MDText.com, Inc.*

2007), they act by secreting WNT9B (Carroll et al., 2005). Other members of the WNT family are also involved: WNT7A is needed for advanced Fallopian tube and uterine development (Parr and McMahon, 1998), whereas WNT5A is essential for distal growth of the Müllerian ducts (Mericskay et al., 2004). WNT signaling cascades seem to be dependent on normal expression of HNF1β in Wolffian ducts (Lokmane et al., 2010). Homeobox genes are also important for Müllerian duct development: HOXA9 is expressed in areas destined to become the Fallopian tube; HOXA10 in the developing uterus; HOXA11 in the primordia of the lower uterine segment and cervix; and HOXA13 in the ectocervix and upper vagina (Du and Taylor, 2004).

The urogenital sinus and the primordia of the external genitalia. Cloaca and urogenital sinus. The urogenital system and the hindgut end in a common chamber, the cloaca, an endoderm-lined cavity covered at its distal boundary by surface ectoderm. This boundary between the endoderm and the ectoderm forms the cloacal membrane. The terminal portion of the hindgut enters into the dorsal region of the cloaca, whereas the allantois enters into its ventral portion. Between the hindgut and the allantois, there is a layer of mesoderm, called the urorectal septum, which progressively grows toward the cloacal membrane between the fourth and seventh week and finally separates the urogenital sinus anteriorly from the anorectal canal posteriorly. By the end of the seventh week, the cloacal membrane ruptures and gives rise to a ventral opening for the urogenital sinus and the anal opening for the hindgut. Between these openings, the tip of the urorectal septum forms the perineal body (Fig. 4).

Labioscrotal swellings and urethral folds. In the third week, mesenchyme surrounds the cloacal membrane, forming the cloacal folds. Cranially to the cloacal membrane, the

folds unite to form the genital tubercle. In 6-week embryos, the ventral portion of the cloacal folds surrounds the opening of the urogenital sinus and forms the urethral folds. Another pair of elevations, the genital or labioscrotal swellings, become visible on each side of the urethral folds. These are connected to the caudal poles of the genital ridges by fibrous bands, which later develop into the gubernaculum testis in males and the round ligament in females (Fig. 4).

Genital tubercle. The genital tubercle emerges as a ventral medial outgrowth just cranial to the urogenital ostium. The genital tubercle gives rise to the phallus, the future penis (male) or clitoris (female). The corpora cavernosa of the phallus and the corpus spongiosum, surrounding the urethra and also forming the glans, arise from the mesodermal tissue in the phallus. Initially they are solid structures, but later they become cavernous owing to the development of vascular spaces. After the corpora cavernosa and glans have differentiated, the ventral surface of the genital tubercle is depressed by a deep furrow, the urethral groove. Endodermal epithelial cells from the urogenital sinus are thought to invade the genital tubercle to form the midline epithelial urethral plate, which lies in the roof of the primary urethral groove and extends to the tip of the phallus (Penington and Hutson, 2002; Blaschko et al., 2012). The prepuce derives from the growth of an ectodermal plate into the superficial part of the phallus. The external genitalia remain undifferentiated up to approximately 9 weeks (Jirásek, 1977) (Fig. 4).

Molecular mechanisms involved in the development of the urogenital sinus and external genitalia. MID1, an ubiquitin-specific regulator of the microtubule-associated catalytic subunit of protein phosphatase 2Ac, is expressed in the urethral epithelium (Dal Zotto et al., 1998). SHH-GLI-BMP4 signaling pathway is also likely to be involved in urogenital epithelium development. P63, also expressed in the urothelium, is a master regulator in epithelial stratification acting via PERP, and its knockout in mice results in excessive urothelial apoptosis (Mahfuz et al., 2013).

ROR2, a putative receptor for WNT5A, is expressed in the genital tubercle and thought to be involved in its development (Schwabe et al., 2004). Homeobox gene products, initially described for their role in limb development, are also necessary for the early formation of the urogenital sinus and the primordia of external genitalia. HOX4 is expressed in the distal trunk and participates in the patterning of the genital tubercle (Dollé et al., 1991). HOXA13 and HOXD13 are also present in the distal trunk and play a role in the development of the genital tubercle, the accessory sex glands, and the smooth muscle layer of the anal sphincter, as well as of the Wolffian and Müllerian ducts (as described above), the lower urinary tract, and the rectum (Warot, 1997). SHH regulates mesenchymally expressed genes *BMP4, HOXD13, FGF10, and PTCH1*, necessary for the outgrowth of the genital tubercle (Haraguchi et al., 2001).

SEX DIFFERENTIATION OF THE GONADS AND GENITALIA

The pioneering experiments on sex developmental physiology in the mammalian embryo accomplished by Alfred Jost in the mid-20th Century (Jost, 1953) established two major concepts: (a) the existence of testes is determinant for male differentiation of the internal and external genitalia, and (b) the testes secrete two discrete factors: androgens, responsible for differentiation of Wolffian ducts, the urogenital sinus, and external genitalia, and a Müllerian inhibitor (now known as anti-Müllerian hormone [AMH]). Therefore, irrespective of their chromosomal constitution, when the gonadal primordia differentiate into testes, all internal and external genitalia develop following the male pathway (Fig. 1). When no testes are present, the genitalia develop along the female pathway. The existence of ovaries has no effect on fetal differentiation of the genitalia.

Gonadal differentiation. Although the importance of the existence of one Y chromosome for the development of the testes, irrespective of the number of X chromosomes present, has been evident since 1959, the identification of the testis-determining factor on the Y chromosome proved unsuccessful, until *SRY* was cloned in 1990 (reviewed by Rey and Josso, 2013). Considerable progress has been made since then, and it has become clear that sex determination is a complex process, regulated by competing molecular pathways in the supporting cell lineage of the bipotential gonad. *SRY* and its related gene *SOX9* are the critical testis-determining genes; however, many genes upstream and downstream of SRY and SOX9 are also essential for normal testis development (reviewed by Eggers et al., 2014).

In brief, during the seventh week in the XY human embryo, the undifferentiated gonads begin to express SRY in the coelomic epithelium somatic cells that will differentiate into Sertoli cells. Together with peritubular cells of mesonephric origin and germ cells, Sertoli cells aggregate to form testicular cords and secrete AMH. Approximately 1 week later, Leydig cells differentiate in the interstitial tissue and secrete testosterone. The mechanisms involved in the regulation of Sertoli and Leydig cell functional differentiation are beyond the scope of this review (see Rey and Grinspon, 2011). The testes descend from their abdominal position to reach the scrotum by the end of the gestation. The action of Leydig-cell–secreted androgens and INSL3, together with the increase in the abdominal cavity pressure induced by the closure of the anterior abdominal wall, is necessary for normal testicular descent (reviewed by Nation et al., 2009).

Ovarian development does not seem to be dependent on a single genetic switch: several early expressed genes seem to be essential for correct ovary development (reviewed by Eggers et al., 2014). However, as already

mentioned, the existence or the absence of ovaries does not modify genital differentiation during fetal life.

Differentiation of the male internal reproductive tract. The mesonephric nephrons and caudal tubules degenerate, but the cranial tubules persist to form the male efferent ducts, connecting the seminiferous cords with the Wolffian duct. Between weeks 9 and 13, the upper portion of the Wolffian duct differentiates into the epididymis. The remaining portion becomes surrounded by smooth muscle and forms the vas deferens, which opens into the urogenital sinus at the level of Müllerian tubercle. The seminal vesicle originates from a dilatation of the terminal portion of the vas deferens in the 12th week. The mesonephric duct ends move close together to enter the prostatic urethra and become the ejaculatory ducts (Fig. 3).

The Wolffian ducts are dependent on androgens for their maintenance and male differentiation; in the absence of androgen action (i.e., in the female fetus or in male fetuses with defective androgen synthesis or androgen receptor activity) Wolffian ducts regress. Testosterone is thought to be the sole factor responsible for Wolffian duct differentiation, acting directly into and down the ipsilateral duct by diffusion. Growth factors and other factors modulating epithelial–mesenchymal interactions are involved in the development of Wolffian derivatives (reviewed by Hannema and Hughes, 2007).

Müllerian regression is the first sign of male differentiation of the genital tract and occurs during the ninth week. Once initiated, the Müllerian duct regression extends cranially and caudally, sparing the cranial tip, which becomes the Morgagni hydatid, and the caudal end, which participates in the organogenesis of the prostatic utricle. Müllerian regression is characterized by a wave of apoptosis spreading along the Müllerian duct (Allard et al., 2000). Adequate levels of AMH and normal AMH receptor signaling are necessary (reviewed by Josso et al., 2013). WNT7A induces AMH receptor expression in Müllerian ducts, essential for AMH sensitivity (Parr and McMahon, 1998).

Differentiation of the female internal reproductive tract. In the absence of androgen action, Wolffian ducts start regressing in the 10th week. The only parts remaining from the mesonephric system are the epoophoron, paroophoron, and Gartner's cyst (reviewed by Rey and Josso, 2013).

In the absence of AMH action, Müllerian ducts persist and differentiate into the Fallopian tubes, the uterus, and the upper part of the vagina. The coelomic epithelial line gives rise to the endometrium, whereas the surrounding mesenchyme differentiates into the myometrium.

As already mentioned, members of the WNT family are involved in normal female tract development: WNT7A in advanced Fallopian tube and uterine development (Parr and McMahon, 1998), and WNT5A in distal growth of the Müllerian ducts (Mericskay et al., 2004).

Differentiation of the urogenital sinus and external genitalia. The urogenital sinus gives rise to the bladder in both sexes, to the whole urethra in the female, and the prostatic and membranous portions of the urethra in the male. The epithelium is of endodermal origin, except for the bladder trigone formed by incorporation of the mesonephric ducts of mesodermal origin. With time, the mesodermal lining of the trigone is replaced by endodermal epithelium so that, finally, the inside of the bladder is completely lined with endodermal epithelium.

The smooth muscle surrounding the bladder and the urethra is derived from visceral mesoderm. At the end of the 10th week, the epithelium of proximal urethra forms several outgrowths that penetrate the surrounding mesenchyme and will give rise to the prostatic buds in the male. In the female, the cranial part of the urethra gives rise to the urethral and paraurethral glands.

Prostate. Male differentiation of the urogenital sinus is characterized by prostatic development and by the repression of vaginal development. Prostatic buds grow into solid branching cords. SOX9 (Thomsen et al., 2008) and FGF10 (Donjacour et al., 2003) play a role in early prostate bud differentiation. Maturation of the prostatic gland is accompanied by development of the prostatic utricle. Two buds of epithelial cells, called the sinoutricular bulbs in the male, develop from the urogenital sinus close to the opening of the Wolffian ducts and grow inward, fusing with the medial Müllerian tubercle to form the sinoutricular cord, enclosed within the prostate gland, which canalizes at 18 weeks to form the prostatic utricle, the male equivalent of the vagina.

Prostate differentiation is dependent on dihydrotestosterone (DHT), a more potent androgen derived from testosterone conversion by the enzyme 5α-reductase or by a more complex pathway (the "backdoor pathway") not involving testosterone synthesis (reviewed by Auchus and Miller, 2012). FGF10 and SHH promote prostate growth by maintaining NOTCH1/HES1 activity, whereas BMP4 and BMP7 signaling from the mesenchyme suppresses prostate branching (Grishina et al., 2005).

Penis and scrotum. During the 9th to 10th weeks, the anogenital distance increases as the first sign of masculinization of the external genitalia (Jirásek, 1977). Subsequently, the labioscrotal swellings (that had arisen in the inguinal region) move caudally and give rise to each hemiscrotum. Fusion of the labioscrotal swellings occurs in a dorsal to ventral fashion and forms the scrotum, which will contain the testis descending from their originally abdominal position.

Like the scrotal swelling, the urethral folds fuse from the posterior to the anterior end, which finally results in the position of the meatus in the tip of the glans penis (Fig. 4). Urethral organogenesis is complete at 14 weeks, yet a physiologic ventral curvature can persist up to the

TABLE 1. *CLASSIFICATION OF DISORDERS OF SEX DEVELOPMENT*

A. Non-endocrine DSD (malformative DSD)
A.I. Defective morphogenesis of the gonadal ducts
Müllerian duct aplasia
Other uterine malformations
Absence of the vasa deferentia
A.II. Defective morphogenesis of the cloaca and urogenital sinus
Cloacal malformations
Exstrophy of the bladder
Prostate defects
A.III. Defective morphogenesis of the primordia of the external genitalia
Aphallia and micropenis
Diphallia and bifid phallus
A.IV. Associated defects in the morphogenesis of the urogenital sinus and the primordia of the external genitalia
Isolated hypospadias
Epispadias
Penoscrotal transposition

B. Endocrine DSD
B.I. Dysgenetic DSD (abnormal gonadal differentiation)
Complete or partial gonadal dysgenesis
Asymmetric gonadal differentiation
Ovotesticular DSD
B.II. Non-dysgenetic DSD
Defective male hormone production or action in 46,XY
Defects of androgen production
Androgen insensitivity (androgen receptor mutations)
Persistent Müllerian duct syndrome: defects of AMH production or AMH receptor mutations
Excess of male hormones in 46,XX
Congenital adrenal hyperplasias
Placental aromatase defects
Adrenal or ovarian tumors
Iatrogenic

sixth month. However, surprisingly, no size difference exists between penile or clitoral size until 14 weeks (Zalel et al., 2001). Maximal phallic growth occurs during the third trimester of fetal life. All these steps are dependent on normal DHT production and androgen receptor function.

The development of the male penile urethra raises controversies: many hold that the proximal urethra forms by fusion of the urethral folds around the urethral plate and the distal urethra arises from an invagination of the apical ectoderm, whereas others maintain that the entire male urethra is of endodermal origin, formed by the urethral plate dorsally and the fused urethral folds ventrally. The seam is remodeled into the tubularized urethra without connection to the epidermis. The ventrally discarded excess epithelial cells migrate into the ventral skin of the penis. Urethral closure during the androgen-dependent phase of penis development is mediated by FGF10, signaling through FGFR2-IIIb (Petiot et al., 2005).

Vagina, clitoris, and labia. At the site where Müllerian ducts reach the urogenital sinus, two solid evaginations, the sinovaginal bulbs, grow out from the pelvic part of the sinus. They proliferate and form a solid vaginal plate that separates the uterus and the urogenital sinus. By the fifth month, the vaginal plate is canalized. The lower end of the vagina slides down along the urethra until the vaginal rudiment opens directly on the surface of the perineum at 22 weeks. Thus, the vagina has a dual origin, with the upper portion derived from the Müllerian ducts and the lower portion derived from the urogenital sinus. The hymen marks the separation between the vagina and the pars phallica of the urogenital sinus which becomes the vestibule (Fig. 4). The whole process requires the lack of action of both AMH (for the development of the upper vagina) and androgens (for the formation of lower vagina independently of the distal urethra). PAX8 (Mittag et al., 2007), LTAP, and VANGL2 (Kibar et al., 2001) are involved in the correct vaginal patterning and opening.

Also in the absence of androgen action, the phallus grows less and forms the clitoris; the urethral folds do not fuse and form the labia minora, whereas the labioscrotal swellings give rise to the labia majora.

Malformative Disorders of Sex Development

DSD are congenital conditions in which chromosomal, gonadal, or genital sex are not coincident. Based on their pathogenesis, DSD may be classified into: (1) malformative (nonendocrine-related) DSD in eugonadal states, wherein abnormal morphogenesis of the genital primordia occurs in early embryonic life; (2) endocrine-related DSD, including (a) dysgenetic DSD in patients with whole gonadal early fetal-onset primary hypogonadism resulting in impaired androgen and AMH production because of abnormal gonadal differentiation, and (b) non-dysgenetic DSD in 46,XY patients, with cell-specific early fetal-onset primary hypogonadism (i.e., Leydig-cell–specific androgen synthesis defects or Sertoli-cell–specific AMH synthesis deficiency) or with end-organ androgen or AMH insensitivity, or patients with an excessive exposure to sex hormone agonists (46,XX fetuses exposed to androgens) or antagonists (46,XY fetuses exposed to antiandrogens) (Table 1).

TABLE 2. *MALFORMATIVE (NONENDOCRINE) DISORDERS OF SEX DEVELOPMENT IN WHICH A GENETIC ETIOLOGY HAS BEEN IDENTIFIED*

Syndrome	Gene mutation	Defect	OMIM[a]
Mayer–Rokitansky–Küster–Hauser	*WNT4*, *LHX1*, *TBX6*	Müllerian derivative malformation or agenesis	277000
MURCS association		Müllerian derivative malformation or agenesis, unilateral renal agenesis, and cervico-thoracic somite dysplasia	601076
RCAD (renal cysts and diabetes)	*HNF1B*	Müllerian aplasia and renal dysfunction	137920
McKusick–Kaufman	*MKKS*	Vaginal atresia	236700
Hand-foot-genital	*HOXA13*	Hypospadias; vagina, uterus, and bladder malformations	140000
Velocardiofacial	Del 22q11.2, *TBX1*	Hypospadias; Müllerian aplasia and renal dysfunction	192430
Cystic fibrosis	*CFTR*	Congenital absence of vasa deferentia	277180
Robinow	*WNT5A*	Aphallia or micropenis	180700
Opitz G/BBB	*MID1*	Hypospadias	300000
Bladder exstrophy and epispadias complex	*PERP*	Epispadias associated to bladder exstrophy	600057

[a]OMIM: Online Mendelian Inheritance of Man (available at: http://www.ncbi.nlm.nih.gov/omim).

In this review, we will address malformative DSD (also called nonendocrine or nonhormonal DSD), that is, those conditions in which the development of the indifferent internal ducts, the urogenital sinus, and/or the primordia of the external genitalia occurring in early embryonic life are impaired, because of an early dysmorphogenetic process not affecting the development of the gonadal ridge. In these cases, no disturbed testosterone or AMH production or action is present (Table 2).

Malformative nonendocrine DSD should be considered when there is inconsistency in the development of the different components of the genitalia. In this review, we classify malformative DSD according to the primordia primarily affected.

DEFECTIVE MORPHOGENESIS OF THE GONADAL DUCTS

Müllerian aplasia, including Rokitansky syndrome. Malformations of the uterus and vagina might result from failure of caudal migration of the Müllerian ducts or failure of canalization of the uterovaginal primordium (Hutson et al., 2014). When agenesis of the Müllerian derivatives is unilateral, the ipsilateral urinary system structures may also be absent, like Mayer–Rokitansky–Küster–Hauser syndrome (MRKH) syndrome (OMIM 277000). The incidence of MRKH syndrome has been estimated as 1 in 4500 female births. The majority of cases seem to be sporadic; however, familial cases have also been described (Morcel et al., 2007). MRKH syndrome is a heterogeneous disorder characterized by uterovaginal atresia in 46,XX girls. Abnormalities of the genital tract may range from upper vaginal atresia to total Müllerian agenesis associated with urinary tract abnormalities and even cervicothoracic somite dysplasia, where uterus agenesis is associated with renal, vertebral, ear, and cardiac defects (MURCS association, OMIM 601076).

MRKH syndrome may be caused by an initial defect of the paraxial and intermediate mesoderm, leading by the end of the fourth week of fetal life to an alteration of the cervicothoracic somites and the pronephric and mesonephric ducts (Morcel et al., 2007). Familial cases support the hypothesis of a genetic cause. The condition seems to be transmitted as an autosomal dominant trait with incomplete penetrance and variable expressivity. Although initially thought to be due to abnormal activation of AMH expression or AMH receptor signaling in the female fetus, no mutations have been found in these genes (Zenteno et al., 2004). Mutations in the *WNT4* gene have been identified in a small subset of MRKH patients with signs of hyperandrogenism (Biason-Lauber et al., 2004). Genome-wide analyses have identified recurrently defective loci in 1q21.1, 17q12, and 22q11.21 (Ledig et al., 2011) and in 16p11.2 and 17q12 (Sandbacka et al., 2013), suggesting that defects in *HNF1B*, *LHX1*, and *TBX6* may be involved in the etiology of Müllerian aplasia or MRKH syndrome. Indeed, Müllerian aplasia has been described in patients with RCAD (renal cysts and diabetes) syndrome (OMIM

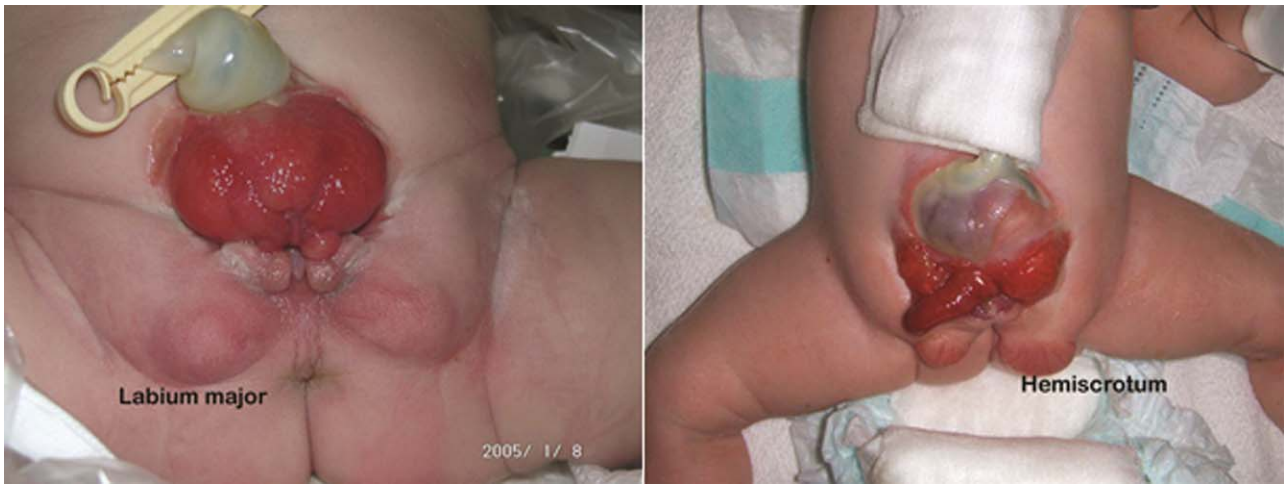


FIGURE 5. *Congenital malformation of the cloacal derivatives hampering the identification of the external genitalia.* **Left:** *bladder exstrophy in a female newborn.* **Right:** *cloacal exstrophy in a male newborn. Note the difficulty in distinguishing the derivatives of the labioscrotal swellings (labia majora and scrotum), urethral foldings, and genital tubercle. Reprinted from: Ebert AK, Reutter H, Ludwig M, Rosch WH. 2009. The exstrophy-epispadias complex. Orphanet J Rare Dis 4:23. doi:10.1186/1750-1172-4–23. ©Ebert et al.; licensee BioMed Central Ltd.*

137920), characterized by renal dysfunction and maturity-onset diabetes of the young type 5 (MODY-5), because of a mutation in the *HNF1B* gene, previously named *TCF2* (Horikawa et al., 1997) and in patients with velocardiofacial syndrome because of a deletion of chromosome 22q11.2 (OMIM 192430). Vaginal atresia has been described in McKusick–Kaufman syndrome (OMIM 236700), probably caused by mutations in the *MKKS* gene (Stone et al., 2000). Malformations of the vagina, uterus, and bladder also occur in females with hand-foot-genital syndrome (OMIM 140000) because of *HOXA13* mutations (Ekici et al., 2013).

Women usually present with primary amenorrhea with normal development of secondary sexual characteristics and normal external genitalia, indicating normal endocrine ovarian function. Treatment of vaginal aplasia results in creation of a neovagina that can allow sexual intercourse.

Other uterine malformations. Lack of fusion of the Müllerian ducts results in duplication of the uterus. In the most severe form, the uterus is entirely double (uterus didelphus), but the condition can be less severe and present as a slightly indented uterus (uterus arcuatus). The uterus bicornis is characterized by the existence of two horns entering a common vagina. In patients with complete or partial atresia of one of the Müllerian ducts (uterus bicornis unicollis with one rudimentary horn), its lumen does not communicate with the vagina, and complications are frequent. If the atresia is bilateral, it is usually associated with atresia of the cervix. A double vagina occurs when the sinovaginal bulbs fail to fuse, and an atresia of vagina results when they do not develop at all.

Obstructed hemivagina and ipsilateral renal anomaly syndrome, formerly known as the Herlyn–Werner–Wunderlich syndrome, is a rare entity characterized by the presence of a uterus didelphys with an obstructed hemivagina caused by a vaginal septum and the association of a renal anomaly (most commonly renal agenesis) ipsilateral to the obstruction (Mandava et al., 2012).

Absence of the vasa deferentia. Unilateral agenesis of the Wolffian duct is usually associated with ipsilateral absence of the kidney and ureter, indicating a defect in early development of mesenophric structures giving rise to the Wolffian duct and the ureteric bud.

Congenital bilateral absence of the vas deferens is responsible for 1% to 2% of cases of male infertility and is present in 95% of patients affected with cystic fibrosis (OMIM 277180), a bronchial and pancreatic disease because of mutations in the cystic fibrosis transmembrane conductance regulator. Whether efferent duct maldevelopment is a primary defect of cystic fibrosis or a secondary degenerative change resulting from obstruction by mucus is not known till now. Isolated absence of vas deferens in otherwise healthy men is often associated with the presence of a single cystic fibrosis transmembrane conductance regulator allele mutation (Chillon et al., 1995). When congenital bilateral absence is associated with renal malformations, cystic fibrosis is unlikely (Anguiano et al., 1992).

DEFECTIVE MORPHOGENESIS OF THE CLOACA AND UROGENITAL SINUS

Cloacal malformations. In cloacal malformations, perineal anatomy may be so affected that the sex of the external genitalia cannot be easily identified (Fig. 5). Although urologic and hindgut anomalies are the major problems to be solved in these cases, sex assignment could also be a serious issue. Karyotype and hormone laboratory evaluations

are useful for establishing gonadal sex and function: hormone levels are within the normal range for genetic sex, indicating that the gonads are not affected. Depending on severity, cloacal malformations may involve the urinary system, pelvis, abdominal wall, genitalia, spine, and anus (Ebert et al., 2009).

Exstrophy of the cloaca. It is a severe ventral body wall defect in which progression and closure of the lateral body wall folds are disrupted. Incidence of the defect is 0.5 to 1 in 200,000 live births, with an overall greater proportion of affected females (Feldkamp et al., 2011). In addition to the closure defect, normal development of the urorectal septum is altered, such that anal canal malformations and imperforate anus occur. Depending on severity, it may also involve malformations of the urinary system, musculoskeletal system, pelvic floor, and spine. Omphalocele is common, and most patients have a single umbilical artery.

Exstrophy of the cloaca may be associated with failure of fusion of the genital tubercle and pubic rami. Furthermore, because the body folds do not fuse, the genital swellings are widely spaced, resulting in defects in the external genitalia. Abnormal partitioning of the cloacal membrane causes displacement of the genital tubercle and, therefore, epispadias. Males may have cryptorchidism, because of the lack of the necessary abdominal pressure for testis descent (Ebert et al., 2009).

Persistence of the cloaca. It is a rare cloacal malformation resulting from a failure in the urorectal septum development with an incidence of 2 in 100,000 births (Warne et al., 2002). The condition is characterized by direct communication between the gastrointestinal, urinary, and genital structures, resulting in a single perineal opening. In many cases, the diagnosis is performed in the prenatal period by sonographic findings as transient fetal ascites, bicystic intraabdominal structure arising from the fetal pelvis (Fallopian tubes), oligohydramnios, and impaired growth. Urinary tract anomalies, such as hydronephrosis, hydroureter, or dysplastic kidneys are the most common secondary anomalies because of urinary outflow obstruction (Warne et al., 2011).

The cloacal dysgenesis sequence is a lethal malformation. The exposure time to urinary outlet obstruction, reduced amniotic fluid volume, and coexistence of severe kidney pathology impairs lung development and neonatal survival. There is absence of anal, genital, and urinary openings with intact perineum covered by smooth skin.

Exstrophy of the bladder. Reported incidence of exstrophy of the bladder varies from 0.5 to 4.6 per 100,000 live births. Unexpectedly, bladder exstrophy is nearly twice as common among male as among female cases, and >70% of the cases are isolated (Siffel et al., 2011). Like the exstrophy of the cloaca, the condition results from a defect in the closure of the anterior abdominal wall, but with normal development of the urorectal septum; consequently, the posterior bladder wall is exposed through a midline defect of the abdomen. The umbilicus is inferiorly displaced and located close to the superior margin of the exstrophic bladder. Genital abnormalities are common in boys and girls who may present epispadias and a small, split phallus or a split clitoris, a bifid uterus, and a duplicate or exstrophic vagina. Bilateral inguinal hernias are palpable in most patients of both sexes. In boys, testes usually remain cryptorchid.

Prostate defects. Nonendocrine defects of prostate development are rare. Congenital anomalies of the prostate (agenesis and hypoplasia) are frequently associated with other anomalies of the urogenital system. Congenital hypoplasia is often associated to prune belly syndrome (Greskovich and Nyberg, 1988). In utero exposure to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin has been shown to cause ventral prostate agenesis in mice by preventing ventral prostatic budding in the embryonic urogenital sinus (Hofkamp et al., 2010).

DEFECTIVE MORPHOGENESIS OF THE PRIMORDIA OF THE EXTERNAL GENITALIA

Aphallia and micropenis. Absence of the phallus or aphallia is a rare disorder with a reported prevalence of 1 in 10 to 30 million live births (Bothra and Jain, 2012). Aphallia can be an isolated disorder in a patient with the rest of the genitalia normally configured. However, it is often associated with other congenital anomalies of the genitourinary, gastrointestinal, and musculoskeletal systems. Associated genitourinary abnormalities are seen in up to 54% of patients (Evans et al., 1999). Aphallia may also be part of a severe form of urorectal septum malformation sequence, where it coexists with severe malformations as imperforate anus, bilateral renal dysplasia, and complete right lung (Gerard-Blanluet et al., 2011).

In XY patients, penile agenesis with a normal scrotum and raphe appears to stem from an isolated malformation of the genital tubercle. There is complete absence of all components of the phallus, namely corpora cavernosa and corpus spongiosum, resulting from nonformation of the genital tubercle or its failure to develop. The urethra can open in any position, from prescrotal to high in the rectum, because the penile urethra fails to develop. As the labioscrotal folds fuse, a normal scrotum and raphe are seen.

Micropenis is defined by a penis length of less than 2.5 standard deviations below the mean, measured along the dorsal surface from the pubis to the tip with the penis stretched to resistance. Micropenis is usually the result of insufficient androgen action, due either to primary or central (hypothalamic–pituitary) hypogonadism or to androgen insensitivity. However, some cases of micropenis are not explained by androgen defects, and are usually called

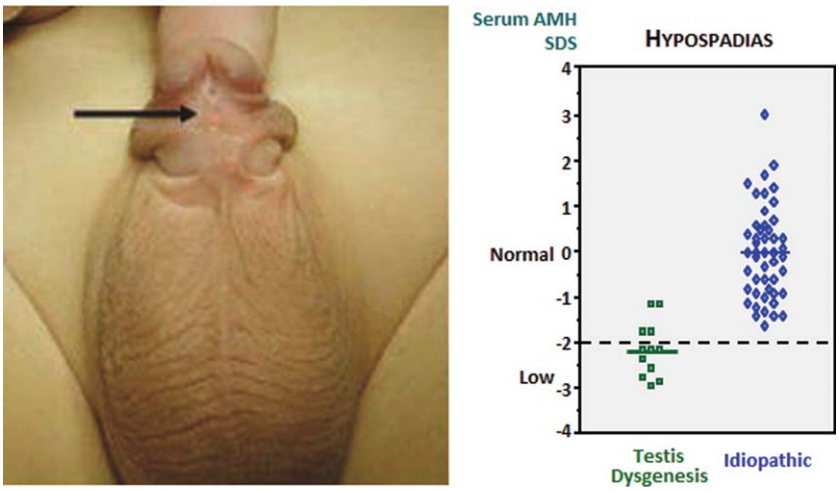


FIGURE 6. *Isolated hypospadias.* **Left:** *Isolated hypospadias in a boy with normal scrotal configuration. Reprinted with permission from: Yucel S, Dravis C, Garcia N, et al. 2007. Hypospadias and anorectal malformations mediated by Eph/ephrin signaling. J Pediatr Urol 3:354–363. ©Journal of Pediatric Urology. Published by Elsevier Ltd. doi:10.1016/j.jpurol.2007.01.199.* **Right:** *In boys with isolated hypospadias, testis hormone secretion (e.g., anti-Müllerian hormone) is normal, as opposed to what is seen in boys with hypospadias because of testicular dysgenesis. Data obtained from Rey et al. (2005).*

idiopathic. Various clinical syndromes may include a micropenis. For instance, the autosomal dominant form of Robinow syndrome (OMIM 180700) because of mutations of *WNT5A* (Person et al., 2010) may present with small or absent penis, associated with short stature, mesomelic limb shortening, hypertelorism, mandibular hypoplasia, and irregular dental alignment.

Clitoris agenesis is a very rare abnormality in XX patients, which has been described in association with agenesis or hypoplasia of labia minora (Martinon-Torres et al., 2000).

The treatment of these conditions varies. Although no treatment is usually attempted in patients with clitoris agenesis, management of isolated penile agenesis has raised many controversies in the last decades. Many were initially prone to feminizing genitoplasty together with early female gender assignment. However, this approach does not take into account potential psychosexual issues arising later in life in individuals who develop a male gender identity. Phalloplasty is a relatively recently developed sophisticated surgical procedure, usually performed in adolescence or adulthood (Callens and Hoebeke, 2014).

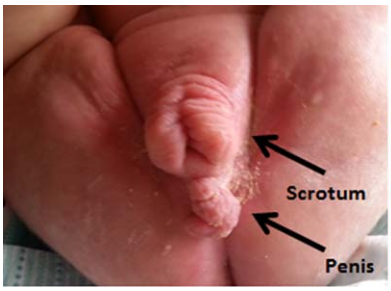
Treatment of micropenis focuses on the attainment of penile size sufficient for the patient to have an appropriate body image, normal sexual function, and standing micturition. The initial attempt consists of exogenous testosterone administration, even if there is no androgen insufficiency. When hormonal treatment does not produce a satisfactory result, phalloplasty has been suggested (Callens and Hoebeke, 2014).

Diphallia and bifid phallus. Diphallia or penile duplication is an extremely rare congenital anomaly, occurring once in every 5.5 million live births (Tirtayasa et al., 2013). True complete diphallia refers to complete penile duplication, each with two corpora cavernosa and a corpus spongiosum. When only one corpus cavernosum is present in each penis, the term bifid phallus applies. It is most frequently associated with an exstrophy of the bladder or other anomalies in the urinary tract because of a malformation of the anterior abdominal wall. From an embryologic point of view, two genital eminences have developed.

ASSOCIATED DEFECTS IN THE MORPHOGENESIS OF THE UROGENITAL SINUS AND OF THE PRIMORDIA OF THE EXTERNAL GENITALIA

Isolated hypospadias. Hypospadias is one of the most prevalent congenital malformations affecting 1 in 125 to 1 in 300 male neonates. The incidence of hypospadias is apparently increasing, with a rate as high as 4% in a recent prospective study (Boisen et al., 2005); this increase has been associated to the potential effect of environmental endocrine disruptors (Main et al., 2010). It is the consequence of an incomplete fusion of the urethral folds present on each side of the urethral groove on the ventral surface of the fetal phallus. Abnormal opening of the urethra occurs along the inferior part of the penis. In rare cases, fusion of the urethral folds fails entirely, and a wide sagittal slits is found along the entire length of the penis and scrotum.

Hypospadias arises between the 8th and 14th week of gestation. Because the closure of the urethral folds is dependent on DHT and its action on the androgen receptor during the first trimester of intrauterine life, most dysgenetic and hormone-dependent forms of DSD present with hypospadias, associated with several features of impaired virilization during fetal life (unfused labioscrotal swellings, small phallus, and cryptorchidism). In these patients, male hormone levels are abnormal: low AMH and androgens in dysgenetic DSD, high AMH and low androgens in steroidogenic synthesis defects, and high AMH and androgens in androgen insensitivity (reviewed by Rey and Grinspon, 2011). Conversely, boys with isolated hypospadias have a low risk of abnormal hormone secretion by the gonads or androgen end-organ defects (Fig. 6) (Rey et al., 2005). These cases could be explained by a genetic



Hormonal laboratory	Patient	Normal values (mean ±SD)*
LH (U/liter)	3.66	4.81 ± 2.19
FSH (U/liter)	0.74	2.30 ± 0.89
Testosterone (ng/dl)	94	76 ± 30
AMH (pmol/liter)	550	427 ± 127

* From Bergadá et al. (2006)

FIGURE 7. *Penoscrotal transposition in a 10-day-old newborn with malformative (nonendocrine) DSD.* **Left***: Note that the penis is dorsally positioned with regard to the scrotum.* **Right***: Endocrine laboratory results showed hormone serum levels within the reference range for age and sex in this patient. Reference values taken from Bergadá et al. (2006).*

or environmentally induced failure in early morphogenetic events responsible for urethral seam formation and development.

Isolated hypospadias, that is, not associated to other genital malformations or gonadal dysfunction, is more frequent in boys born preterm, small for gestational age or with low birth weight, or conceived with fertility treatments (Woud et al., 2014). The underlying pathogeny is poorly understood (Yucel et al., 2007).

Isolated hypospadias also exists as part of polymalformative syndromes. In the X-linked Opitz G/BBB syndrome (OMIM 300000), hypospadias coexists with hypertelorism, cleft lip/palate, laryngo-tracheo-esophageal abnormalities, imperforate anus, developmental delay, and cardiac defects. In these patients, mutations have been described in *MID1*, impairing its ubiquitin ligase activity and leading to a marked accumulation of protein phosphatase 2A, finally resulting hypophosphorylation of microtubule-associated proteins (Dal Zotto et al., 1998; Trockenbacher et al., 2001).

Single gene mutations have remained elusive in the study of patients with isolated hypospadias not associated with other polymalformations, until recent genome-wide association analyses have identified 18 genomic regions explaining 9% of the liability to developing hypospadias. In the identified regions, genes with key roles in embryonic development are present; for example, *DGKK* (van der Zanden et al., 2011), *HOXA4*, *IRX5*, *IRX6*, and *EYA1* (Geller et al., 2014).

Management of hypospadias includes surgical correction (Mouriquand et al., 2011; Springer and Baskin, 2014). Because hypospadias is usually associated with penile chordee, the surgical technique initially requires the correction of the chordee, followed by urethroplasty and meatoplasty. Poor cosmetic results and urethral fistula are the most common complications, followed by urethral stricture (Mouriquand et al., 2011).

Epispadias. Epispadias is a rare abnormality with an incidence of 2.4 per 100,000 live births ([No author listed], 1987). In epispadias the urethral meatus is found on the dorsum of the penis. Epispadias is produced by failure of the urethral plate to tubularize on the dorsum with the defect ranging from a glandular to a penopubic location. Although epispadias may occur as an isolated defect, it is most often associated with exstrophy of the bladder and abnormal closure of the ventral body wall, a condition known as the bladder exstrophy–epispadias complex (Ebert et al., 2009). A recent Genome-Wide Expression Profiling study suggests a possible involvement of the P63-related protein PERP, previously named THW, in the pathogenesis of the bladder exstrophy–epispadias complex (Mahfuz et al., 2013).

Urinary incontinence seems to be the main clinical symptom, because of the degree of involvement of the urinary sphincter. In most distal epispadias, involuntary urine loss is not observed, whereas in complete epispadias urine is dripping permanently through the meatus in both sexes. Distal epispadias might be overlooked at birth, especially in girls. Then diagnosis may be recognized as late as at school age, because of urinary incontinence.

Penoscrotal transposition. Penoscrotal transposition is a rare malformation (Burgos et al., 2012) in which there is a total or partial exchange of position between the penis and scrotum occurs (Fig. 7). The embryologic causes are not well understood. It may be due to a failure in the caudal migration of labioscrotal folds because of an abnormal position of the genital tubercle during the fourth and fifth gestational weeks. The condition can be associated with other anomalies, such as urogenital, lumbosacral, and anorectal malformations (Parida et al., 1995).

When not due to endocrine-related DSD, penoscrotal transposition cases are mostly sporadic, although family history was recorded in approximately 10% of reported cases (Pinke et al., 2001). In these cases, the existence of consanguinity suggests a possible autosomal recessive pattern. Surgical correction is needed, and different techniques have been proposed (Pinke et al., 2001; Sunay et al., 2009).

Concluding remarks

The birth of a child with ambiguous genitalia is a stressful situation. Congenital malformations of the genitalia are most frequently because of androgen deficiency in XY individuals or to androgen excess in XX patients. However, in a nonnegligible number of cases, no endocrine etiology can be found: gonadal function is normal and coincident

with chromosomal sex. Malformative DSD should be considered as a diagnosis in these cases. Although increasing trends in the incidence of malformative DSD is suggestive of environmental etiologies underlying the condition, new high-throughput genetic/genomic technologies are unraveling disrupted loci/genes as potential causes.

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